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REMARKS/ARGUMENTS

The claims remain as 1 and 8 and 10-14.

Claim 7 is amended to recite the excipient as suggested.

Claim 8 is similarly amended.

Claim Objectives

Reconsideration and withdrawal of the objection to Claim 7 is requested since the claim is now amended as suggested.

Claim Rejections – 35 U.S.C. § 112

Reconsideration and withdrawal of the rejection of Claims 8 and 10 under 35 U.S.C. § 112, first paragraph, as failing to comply with the enablement requirement, for the stated reasons that the claim(s) contains subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to use the invention, citing *In re Wands*, 8 USPQ2d 1400 (1988).

Concerning the points itemized in the Wands decision, the following is stated.

1. The invention of Claim 10 relates to the use of imidazole characterized by among other features, the presence of a sulfonamide grouping, as a method of “preventing or treating a disease in a patient treatable with a pharmaceutical compound having hypoglycemic activity,” the disease being one of those specified.

Claim 8 names the compound and simply itemizes its uses.

The invention is thus in the pharmaceutical field.

2. Concerning the state of the prior, this is continuing to seek advances in the treatment of the diseases named and pharmaceuticals containing a sulfonamide group (as glipizide and tolbutamide) or thiazolidinedione grouping are known as oral hypoglycemic

agents, please see the attached pages 1374 and 1375 of Remington, The Science and Practice of Pharmacy, Ed. , Gennard , 20<sup>th</sup> Ed – 2000.

The existence and effectiveness of hypoglycemic agents is therefore well established.

3. Concerning the matter of predictability in this art, the following is offered.

The U.S. Patent Office has allowed U.S. Patent 6,242,474 over which the subject application has been rejected for double patenting, which contains in its Claim 9 the following:

9. A method for preventing or treating impaired glucose tolerance, diabetes, diabetic complications, syndrome of insulin resistance, polycystic ovary syndrome, hyperlipidemia, atherosclerosis, cardiovascular disorders, hyperglycemia, and hypertension comprising administering to a patient in need thereof an effective amount of a heterocyclic aromatic ring compound represented by formula (I):

wherein formula (I) is a generic formula.

Clearly, sufficient predictability has been recognized by the U.S. Patent Office.

Please also note that in Claim 10, all the diseases recitations are limited to versions “treatable with a pharmaceutical compound having hypoglycemic activity.”

The following establishes that treatment predictability in regard to such diseases exists. Copies of the citations are attached.

J.A. Cornicelli, Atherosclerosis, 1997, 2(2), 43-49

This publication is entitled “Diabetes, Insulin Resistance, Hyperinsulinemia and Vascular Disease: A Therapeutic Challenge for the 90s”.

This publication teaches that Troglitazone (please see the Remington text, pg. 1375), which lowers the blood sugar level, may be important for preventing the onset of diabetes in obese individuals with impaired glucose tolerance (page 46, right column, lines 8-11). In Applicants’ view, the compound of the present invention which lowers blood sugar level is easily expected to be effective for the prevention of diabetic complications, insulin resistance

syndrome etc. from the teachings of this publication. This publication also discloses that Troglitazone is effective for hypertriglyceridemia and hypertension (page 45, column 2, the central paragraph penultimate sentences).

Endocr Pract, 2001, 7(4), 279-86

This publication teaches that Troglitazone will be effective for polycystic ovary syndrome. The Abstract is submitted.

Arterioscler Thromb. Vasc. Biol., 2001, 21(12), 1891-5.

This publication teaches that TZDs (thiazolidene diones) improve cardiovascular risk factor and delays the atherosclerotic process. (The Abstract is submitted).

Arch Dermatol, 2000, 136(5), 609-16

This publication teaches that Troglitazone is effective for psoriasis, i.e., one of the skin disorders related to an anomaly of differentiation of epidermic cells. (Abstract submitted).

Endocrinology, 1999, 140(11), 5060-5

This publication teaches that thiazolidinedione derivatives having an antidiabetic activity are effective for inhibition of bone resorption. Therefore, it is easily expected that these derivatives will be effective for osteoporosis. (Abstract submitted).

U.S. 6,353,009

This USP teaches that an insulin sensitivity enhancer has an ability to treat and/or prevent hyperuricemia.

Kirk-Othmer Encyclopedia of Chemical Technology, 4<sup>th</sup> Ed., 1995, page 675 states that Diazoxide exhibits hypoglycemic and antihypertensive effects.

Please note the following quotations:

A body of evidence is accumulating relating hyperinsulinemia, insulin resistance and vascular disease (Cornicelli, page 43, column 1, penultimate sentence in the first paragraph).

The action of this compound (troglitazone) may also be important in preventing the onset of diabetes in obese individuals with impaired glucose tolerance (Cornicelli, page 46, column 1, sentence at line 8).

Cornicelli states, referring to troglitazone/a thiazide)

[64]. Treatment with this agent completely prevented the development of insulin resistance, hypertension and hypertriglyceridemia in fructose-fed rats [65]. (Cornicelli, page 45, penultimate sentence in next to last paragraph).

Current evidence suggests that the use of insulin-sensitizing agents in patients with polycystic ovary syndrome not only improves their sensitivity to the effects of insulin on glucose and lipid metabolism but also ameliorates clinical and biochemical manifestations of hyperandrogenism and increases rates of ovulation. (Endoc. Pract. 2001, 7(4), 279-86), Abstract, penultimate sentence.

Thiazolidinediones (TZDs) are oral insulin sensitizers in broad clinical use that enhance insulin-stimulated glucose uptake into skeletal muscle. TZDs can also improve cardiovascular risk factors and exert direct effects on vascular cells to potentially retard the atherosclerotic process. (Arterioscler Thromb. Vasc. Biol., 2001, 3<sup>rd</sup> and 4<sup>th</sup> sentences of Abstract)

All patients' psoriasis improved substantially during troglitazone therapy. (Arch Dermatol. 2000, below center of Abstract stating results of a test on normoglycemic patients.)

Thus, TZDs may suppress bone resorption in diabetic patients and prevent bone loss. (Endocrinology, 199, last sentence of Abstract).

Claim 1 of USP 6,353,009, reads as follows:

1. A method for the treatment or prevention of hyperuricemia in a human in need thereof, which comprises administering to said human an amount of insulin sensitivity enhancer effective to reduce or prevent hyperuricemia.

The Kirk-Othmer citation includes the following (page 675, last paragraph, first sentence)

Diazoxide [364-98-7] (3-methyl-7-chloro-1,2,4-benzothiadiazine, 1,1-dioxide) is a non-diuretic thiazide used for its

hyperglycemic actions when given orally (Proglycem) and for its antihypertensive effects when given intravenously (Hyperstat IV.)

3. The predictability or lack thereof in the art.

Based upon the above literature citations, it would appear that sufficient predictability exists to sustain Applicants' claims.

4. The amount of direction or guidance present

For pharmaceutical applications, it would appear that the disclosure (page 21, paragraph at line 5) of preparation types for therapeutic administration as well as dosage is of the nature that has been accepted as sufficient in the past.

5. The presence or absence of working examples.

The application is replete with Examples for preparing the compounds. The blood sugar level depressing activity in mice, is demonstrated in Experimental Ex. 1, pages 19-21 for a representative compound, that of Example 11.

6. The breadth of the claims

The claims are limited in the sense that they specify the compound as one requiring the presence of a sulfonamide group and a diazide group, substituted as specified, and linked as specified through a defined linking group L.

The diseases that are treatable are listed and all qualified by "in a patient treatable with a compound having hypoglycemic activity", in the method Claim 10.

Claim 8 (the composition recited as a preparation), merely limits the preparation as one used for the recited purpose.

7. The quantity of experimentation needed

The various citations submitted established that the quantity of experimentation needed is known and acceptable in this art.

8. The level of skill in the art

This is obviously very high.

In view of the above, it would appear that the criteria set forth in the In re Wands decision have been satisfied.

All the diseases recited in the method Claim 10 are limited by the common thread “disease in a patient treatable with a pharmaceutical compound having hypoglycemic activity”.

As for the composition Claim 8, this simply lists possible uses. The composition is infringed by a non-authorized sale whether or not so used.

Favorable reconsideration is therefore solicited of the stated rejection.

Reconsideration and withdrawal of the rejection of Claims 2, 3 and 5 under 35 U.S.C. §112, second paragraph for the stated reasons are requested.

The Official Action, page 8, states:

Claim 2 does not further limit claim 1 {see definition (10) under variable  $R^6$ }. Compounds 30 and 31 in claim 5 are not embraced by claim 1 (see the  $R^1$  definition and the possible substituents).

In support of that conclusion, the Official Action states, page 10,

Nothing in the original filed claims or the instant specification would lead, suggest, or teach to one skilled in the art to substitute any of the claimed substituents of definitions (1)-(11), under  $R^1$  in claim 1, on the “alkyl” portion of the “alkylaryl” (which is embraced by the definition of “aryl”) instead of the aryl portion of the “alkylaryl”.

In response, Applicants point out that formula IA of Claim 2, which also appears on page 11 of the specification serves in fact, among other purposes, as a disclosure of just such substitution of claimed substituents under  $R^1$  on the alkyl portion of the “alkylaryl” embraced by disclosed definition of “aryl”.

In formula (IA) unsubstituted aryl appears as the benzene ring in the depicted phenyl (radical substituted by chlorine (halogen)) at the ortho position. Since only o-chloro aryl(phenyl) is specifically depicted in formula (IA), substituents identified by  $R^6$  must be

separately named. They are indeed so separately named as in recitations (4), which names “halo(lower)alkyl”, and (10), which names “lower alkyl optionally substituted by aryloxy”.

Compounds 30 and 31 of Claim 5 are within the scope of Claim 2 and therefore within the scope of Claim 1.

All that is involved is a change in terminology, with no change in substance.

Formula (IA) thus specifically names the substituents in question, “halo” in substituent (4) and “aryloxy” in substituent (10) on the alkyl portion of “alkyl substituted aryl” included by definition within the term “aryl”. This appears also clearly in Preparation 27-1, page 43 where the grouping “-4-[phenoxymethyl]benzene” appears.

Concerning the quotations from the specification, bridging pages 9 and 10 of the Official Action, those portions of the specification relate to exemplification of “alkyl” per se. Substitution is dealt with elsewhere, as in the paragraph bridging pages 5 and 6 and quite generally in the following quotation at page 11, line 18.

When the above-mentioned substituents are substituted, the number of the substituents is preferably 1 to 4, unless particularly specified.

The above is immediately followed by formula (IA).

In principle, Applicants' position is exemplified by reference to nomenclature relating to substitution on toluene.

(1) (chloromethyl)benzene and (2)  $\alpha$ -chlorotoluene are the same compounds and (3) benzyl chloride is a third name.

The first name is used when the benzene portion of the aromatic compound toluene is written out, as in Claim 2 and Preparation Example 27-1. The second name is suitable where the benzene ring is implied by the generic terminology, as in Claim 1. The third is suitable when the  $C_6H_5-CH_2-$  grouping is to be identified.



Accordingly, compounds subgenerically named as in Claims 2 and 3, and specifically named, as in Claim 5, are within the scope of Claim 1 and limit it as applied to e.g., compound (30) in Claim 5. Another less elegant name for the grouping in issue is  $\alpha$ -phenoxy-p-tolylmethyl-.

In summary, it is Applicants' position

(1) that examples of substitution of the alkyl group on an alkyl substituted aryl moiety (within the disclosed broad definition of aryl) are disclosed.

(2) that Claims 2, 3 and 5 are within the scope of Claim 1.

Claim 3 would appear to be free of the stated criticism.

It is added that Applicants should not be faulted for precisely defining what they intend by "aryl". Their definition conforms to that which is standard in the chemical art, note the definition of "aryl" in Webster's Third New International Dictionary 1993, pg. 125).

ar-yl 1: a univalent aromatic radical (as phenyl or tolyl) derived from an arene by removal of one hydrogen atom from a carbon atom of the nucleus-

The consultant for Chemistry was Patterson, former editor of Chemical Abstract.

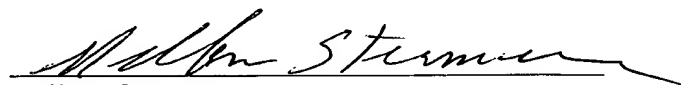
Favorable reconsideration is solicited.

Respectfully submitted,

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**2 0** T H E D I T I O N

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# Remington: The Science and Practice of Pharmacy

**ALFONSO R GENNARO**

Chairman of the Editorial Board  
and Editor

**OBLON, SPIVAK, McCLELLAND,  
MAIER & NEUSTADT P.C.**

**Table 77-5. Oral Hypoglycemic Agents**

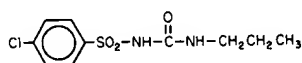
DRUG	COMMENTS
<b>First-generation sulfonylureas</b>	
Tolbutamide	Short acting (6–12 hr)
Tolazamide	Similar to tolbutamide; intermediate acting (10–14 hr); has active metabolites
Acetohexamide	Similar to tolbutamide; intermediate acting (12–24 hr); has active metabolite
Chlorpropamide	Long acting (up to 60 hr); ADH-like action, more serious toxicities
<b>Second-generation sulfonylureas</b>	
Glyburide	More potent than first-generation drugs; effects persist for 24 hr
Glipizide	Shorter half-life (2–4 hr); available as extended-release preparation
Glimepiride	Similar to glyburide and glipizide; more potent; longer half-life (5 hr)
<b>Biguanides</b>	
Metformin	Actions do not involve stimulation of insulin secretion, some GI effects; rarely lactic acidosis
<b>Meglitinides</b>	
Repaglinide	Similar in action to sulfonylureas; short acting (half-life = 1 hr)
<b>Thiazolidinediones (Glitazones)</b>	
Troglitazone	Decreases insulin resistance by effects on target organs
Rosiglitazone	Similar to troglitazone
Pioglitazone	Similar to troglitazone

diated by a competitive, reversible inhibition of pancreatic  $\alpha$ -amylase membrane-bound intestinal  $\alpha$ -glucosidase hydrolase enzymes. Acarbose is metabolized exclusively within the GI tract, principally by intestinal bacteria but also by digestive enzymes. The small fraction of active drug that is absorbed is excreted completely by the kidneys.

The most commonly observed side effects are mild-to-moderate GI effects such as flatulence (77%), diarrhea (33%), and abdominal discomfort (21%) that generally diminish in frequency and intensity with time.

### CHLORPROPAMIDE

**Benzenesulfonamide, 4-chloro-N-[(propylamino)carbonyl]-, Diabinese**



1-[(p-Chlorophenyl)sulfonyl]-3-propylurea [94-20-2]  
 $C_{10}H_{13}ClN_2O_3S$  (276.74).

**Preparation**—p-Chlorobenzenesulfonamide undergoes addition to propyl isocyanate by warming a solution of equimolar quantities of the two reactants.

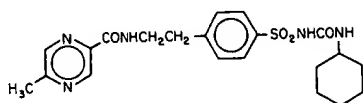
**Description**—White, crystalline powder, with a slight odor; melts between 125° and 129°.

**Solubility**—Practically insoluble in water; soluble in alcohol; sparingly soluble in chloroform.

**Comments**—An oral hypoglycemic agent with actions and uses essentially the same as those of *Tolbutamide*. As with tolbutamide, its use is limited to patients with stable, mild to moderately severe diabetes mellitus who still have some residual pancreatic  $\beta$ -cell function. Refractoriness sometimes develops. Elimination is about 80% hepatic. The half-life (25 to 60 hr) and duration of action (about 24 to 48 hr) are much longer than those of tolbutamide. The side effects are of the same type as with tolbutamide but have a somewhat higher incidence, namely, about 6%, half of which are cutaneous. It increases the endogenous release of vasopressin (ADH) and thus causes water retention with resultant hyponatremia and hypo-osmolality. This action is used in the treatment of central diabetes insipidus. Patients under treatment with this drug have a disulfiram-like intolerance to alcohol. It is contraindicated in the presence of renal glycosuria because of the possibility of fatal hypoglycemia.

### GLIPIZIDE

**Pyrazinecarboxamide, N-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-5-methyl-, Glucotrol**



[29094-61-9]  $C_{21}H_{27}N_5O_3S$  (445.54).

**Preparation**—By the condensation of 4-[2-(5-methyl-2-pyrazinecarboxamido)ethyl]benzenesulfonamide and cyclohexyl isocyanate; *Arzneimittel-Forsch* 1971; 21:200.

**Description**—White, odorless powder;  $pK_a$  5.9; melts about 205°.

**Solubility**—Insoluble in water or polar solvents; freely soluble in dimethylformamide or fixed alkalies.

**Comments**—A sulfonylurea used to treat Type 2 diabetes mellitus that is 100 times more potent than tolbutamide in evoking pancreatic secretion of insulin. It differs from other oral hypoglycemic drugs in that tolerance to this action apparently does not occur. It also up-regulates insulin receptors in the periphery, which seems to be the primary action. It is thought not to have a direct effect on glucagon secretion. It is mildly diuretic. It has a special status in the treatment of non-insulin-dependent diabetes mellitus because it is effective in many patients who are resistant to all other oral hypoglycemic drugs. It differs from other oral hypoglycemic drugs in that it is more effective during eating than during fasting.

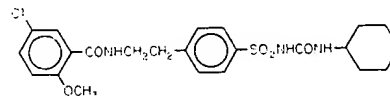
Adverse effects have been reported to occur in as few as 3% and as many as 12%. GI effects, such as nausea, heartburn, diarrhea, constipation, vomiting, and colic, are the most common and occur with a frequency of 1.7 to 3.7%. Rashes occur in 0.5 to 1.5%. Mild headache, drowsiness, asthenia, and dizziness occasionally occur. Minor episodes of hypoglycemia can occur.

Peroral absorption is almost complete. It is rapid (about 30 min) if the stomach is empty, but 1.2 to 3.5 hr is required in the presence of food.

About 98% is bound to plasma albumin by nonionic forces. The volume of distribution is about 0.16 L/kg. About 90% is metabolized in the liver to several inactive metabolites. The half-life is about 2 to 4 hr. The duration of action is 10 to 16 hr.

### GLYBURIDE

**Benzamide, 5-chloro-N-[2-[4-[[[(cyclohexylamino)carbonyl]amino]sulfonyl]phenyl]ethyl]-2-methoxy-, DiaBeta; Glynase Pres Tab; Micronase**



Glybenclamide [10238-21-8]  $C_{23}H_{28}ClN_3S$  (494.00).

**Preparation**—See *Arzneimittel-Forsch* 1966; 16:640; CA 66:65289h.

**Description**—White to off-white crystalline powder; melts about 170°;  $pK_a$  5.3.

**Solubility**—Sparingly soluble in water or ether; 1 g dissolves in 330 mL of alcohol or 36 mL of chloroform.

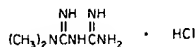
**Comments**—A sulfonylurea hypoglycemic drug used for Type 2 diabetes mellitus. It is 200 times as potent as tolbutamide in evoking the release of insulin from the pancreatic islets. It is more effective in suppressing fasting than postprandial hyperglycemia. Like glipizide, it is mildly diuretic.

The incidence of adverse effects is reported to be 3.6%. GI side effects, such as nausea, anorexia, vomiting, heartburn, diarrhea, constipation, and abdominal pain, occur with a frequency of about 2%. Pruritus and rashes may occur. Cholestatic jaundice and eosinophilia have been reported. Transient leukopenia sometimes occurs. A disulfiram-like reaction to alcohol may occur. Hypoglycemic episodes sometimes are prolonged and severe, and deaths have resulted.

About 90% of oral drug is absorbed from an empty stomach. The absorption time is 1.5 to 2 hr. Food decreases absorption, some fibers decreasing it by as much as 50%. About 97% is bound to plasma albumin as a weak-acid anion and, hence, is susceptible to displacement by many weak acid drugs. Elimination is by hepatic metabolism. The half-life is 1.5 to 5 hr. The duration of action is 24 hr. The micronized tablets are not strictly bioequivalent to nonmicronized tablets in their onset of action or half-life.

## METFORMIN HYDROCHLORIDE

**Imidodicarbonimidic diamide, *N,N*-dimethyl-, monohydrochloride;  
Glucophage: Metiguanide**



[657-24-9]  $C_4H_{11}N_5 \cdot HCl$  (165.67).

**Preparation**—By the reaction of dimethylamine hydrochloride with dicyandiamide to yield the base; *J Am Chem Soc* 1959; 81: 2220, 2728.

**Description**—White crystals melting about 230°

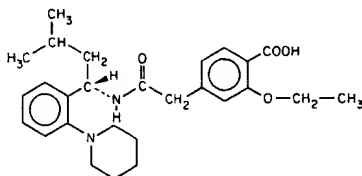
**Solubility**—Soluble in water or alcohol; practically insoluble in ether or chloroform.

**Comments**—An oral antihyperglycemic drug used in the management of Type 2 diabetes mellitus. It may be used as monotherapy or as an adjunct to diet or a sulfonylurea to lower blood glucose levels. It improves glucose tolerance by lowering both basal and postprandial glucose. Its mechanism of action is distinct from that of sulfonylureas and does not result in hypoglycemia. It decreases hepatic glucose production, decreases intestinal absorption of glucose, and improves insulin sensitivity by increasing peripheral glucose uptake and utilization. Oral bioavailability under fasting conditions is 50 to 60%, and food decreases the extent of absorption. The drug is excreted unchanged in the urine and does not undergo hepatic metabolism.

Adverse effects are primarily GI symptoms such as diarrhea, nausea, vomiting, abdominal bloating, flatulence, and anorexia. A rare but serious toxicity that can occur with metformin is lactic acidosis.

## REPAGLINIDE

***p*-Toluic acid, (+)-2-ethoxy- $\alpha$ -[[(*S*)- $\alpha$ -isobutyl-*o*-piperidino-benzyl]carbamoyl]-. Prandin**



[135062-02-1]  $C_{27}H_{36}N_2O_4$  (452.59).

**Preparation**—See Int Pat Appl WO 93 00,337 (1993).

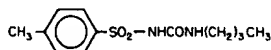
**Description**—White powder melting about 129°.

**Comments**—An oral hypoglycemic agent that is a meglitinide (the nonsulfonylurea moiety of glyburide) that is used in the management of Type 2 diabetes mellitus. It acts by stimulating insulin secretion by binding to and inhibiting the ATP-dependent potassium channels in the  $\beta$ -cell membrane, resulting in an opening of calcium channels. It is absorbed rapidly and completely from the GI tract and is completely metabolized in the liver by oxidative biotransformation and direct glucuronidation. It has a short half-life in the bloodstream of 1 hr. The hepatic cytochrome P-450 system 3A4 is involved in the metabolism of repaglinide, which may be inhibited by some drugs including ketoconazole, miconazole, and erythromycin.

The most common adverse effect is hypoglycemia. The incidence of serious cardiovascular events (3 to 4%) is similar to that with glyburide and glipizide in comparative trials.

## TOLBUTAMIDE

**Benzenesulfonamide, *N*-[(butylamino)carbonyl]-4-methyl-, Orinase**

1-Butyl-3-(*p*-tolylsulfonyl)urea [64-77-7] C<sub>12</sub>H<sub>18</sub>N<sub>2</sub>O<sub>3</sub>S (270.35).

**Preparation**—Toluene is treated with chlorosulfonic acid and the resulting *p*-toluenesulfonyl chloride is converted into *p*-toluenesulfonamide by interaction with ammonia. Condensation of the sulfonamide with ethyl chloroformate in the presence of pyridine or another suitable

basic catalyst produces ethyl *N*-*p*-toluenesulfonylcarbamate. Aminolysis with butylamine in ethylene glycol monomethyl ether solutions yields tolbutamide.

**Description**—White, or practically white, crystalline powder; slightly bitter and practically odorless; melting range is 126 to 132°.

**Solubility**—Practically insoluble in water, soluble in alcohol or chloroform.

**Comments**—An oral hypoglycemic drug. It is useful in the treatment of selected cases of non-insulin-dependent diabetes mellitus. To respond, patients must have some remaining functional islet  $\beta$ -cells that can be stimulated by the drug. Patients who require more than 40

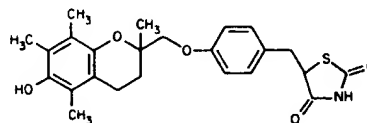
Units of insulin/day generally will not respond to this drug. In diabetic patients the peak effect is reached in 5 to 8 hr. The duration of action is usually 6 to 12 hr (average plasma half-life, 5.6 hr but longer in elderly patients; dose-dependent kinetics occur with high doses), so that two daily doses are required in most patients. The hypoglycemia induced by even high doses of the drug is generally not as severe as can be induced by insulin; hence, the incidence of acute hypoglycemic reactions is lower with tolbutamide; however, severe, refractory hypoglycemia sometimes does occur. Refractoriness to it sometimes develops.

Toxic effects include diarrhea, nausea, vomiting, abdominal cramps, weakness, headache, tinnitus, paresthesias, allergic reactions (pruritus, erythema multiforme, maculopapular rash, all usually transient), photosensitivity, and alcohol intolerance. Water retention and hyponatremia may result from enhancement of ADH (vasopressin) release. Cholestatic jaundice may occur (rarely), and the drug is contraindicated in the presence of liver damage. Rare leukopenia, thrombocytopenia, pancytopenia, and agranulocytosis occur. Hypoglycemic reactions are rare. It is contraindicated in nondiabetic patients with renal glycosuria and liver disease.

The sulfonylureas interact with a number of drugs. The following substances increase the hypoglycemic activity of sulfonylureas: dicumaryl, phenylbutazone, oxyphenbutazone, several sulfonamides, chloramphenicol, large doses of salicylates, monoamine oxidase inhibitors (MAOIs), clofibrate, anabolic steroids, fenfluramine, guanethidine,  $\beta$ -adrenoreceptor-blocking drugs, and alcohol.

## TROGLITAZONE

**2,4-Thiazolidinedione, (±)-5-[[4-[3,4-dihydro-6-hydroxy-2,5,7,8-tetra-methyl-2H-1-benzopyran-2-yl)methoxy]phenyl)methyl]-, Rezulin**



[97322-87-7]  $C_{24}H_{27}NO_3S$  (441.55).

**Preparation**—See US 4,572,912 (1985).

**Description**—White to yellowish with faint characteristic odor, melting about 185°.

**Solubility**—Soluble in DMF, anhydrous ethanol, acetonitrile, or ether; practically insoluble in water.

**Comments**—An oral drug that improves the responsiveness to insulin, when patients with Type 2 diabetes mellitus have problems of insulin resistance, by a unique mechanism of action compared with those of other drugs available. It is currently only approved for use with insulin. The drug decreases blood glucose in diabetic patients with hyperglycemia by improving target organ response to insulin. In the presence of exogenous or endogenous insulin, the drug decreases hepatic glucose output, increases insulin-dependent glucose uptake and use by skeletal muscle, and increases glucose uptake and decreases fatty acid output in adipose tissue. Troglitazone binds to nuclear receptors called peroxisome proliferator-activated receptors (PPARs) that regulate transcription of a number of insulin-responsive genes critical to glucose and lipid metabolism. It is not an insulin secretagogue.

It is absorbed rapidly upon oral administration, and food increases the extent of absorption by 30 to 80%; therefore, it is taken with meals to enhance systemic availability. The drug is highly bound (>99%) to serum albumin. It is metabolized in the liver to several inactive compounds including a sulfate conjugate, the major metabolite, and primarily eliminated in the feces. The hepatic cytochrome P-450 that is involved in metabolism is CYP3A4, so drug interactions can be expected when used in combination with drugs such as oral contraceptives, terfenadine, and others that are metabolized by the same enzyme.

The incidence of side effects is relatively low, including headache and respiratory infections. It is important to monitor liver function by measuring increases in serum levels of aminotransferases that occur in 1 to 2% of patients. In addition, serious hepatotoxicity may occur in rare

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occurring in the form of tears and dark-colored masses, having a strong odor and taste, and formerly used in medicine as an antispasmodic



KIRK-OTHMER

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and is undergoing clinical investigation in the United States. Because of a different mechanism of action, biguanides might be used instead of or in combination with sulfonylureas.

**Phenformin.** Phenformin hydrochloride [834-28-6] (1-phenethylbiguanide, *N*-(2-phenylethyl)imidodicarbonimidic diamide), is a white to off-white odorless crystalline power having a bitter taste. The melting point is 175–178°C. It is freely soluble in water and alcohol, and practically insoluble in chloroform, ether, and hexane. Its pH in solution is 6.0–7.0.

**Metformin.** Metformin [657-24-9] (1,1-dimethylbiguanide), mol wt 129.17, forms crystals from propanol, mp 218–220°C, and is soluble in water and 95% ethanol, but practically insoluble in ether and chloroform. Metformin, an investigational drug in the United States, does not increase basal or meal-stimulated insulin secretion. It lowers blood glucose levels in hyperglycemic patients with Type II diabetes but has no effect on blood glucose levels in normal subjects. It does not cause hypoglycemia. Successful metformin therapy usually is associated with no or some weight loss.

Clinical studies have shown that metformin is as effective as chlorpropamide, tolbutamide, or gliclazide in lowering fasting and post-prandial hyperglycemia in patients with newly diagnosed Type II diabetes. However, metformin treatment produces a modest mean weight loss of 1–2 kg as compared with a mean weight gain of 1.5–5.0 kg produced by sulfonylurea therapy. Therapy using metformin results in better glycemic control in about 80% of both obese and nonobese patients having newly diagnosed Type II diabetes. Reported rates of primary failure with metformin are about 12%, and those of secondary failure are about 5%.

**Inhibitors of  $\alpha$ -Glucosidase.** Acarbose [56180-94-0] (*O*-4,6-dideoxy-4-[[[1*S*-(1 $\alpha$ ,5 $\beta$ ,6 $\alpha$ )]-4,5,6-trihydroxy-3-(hydroxymethyl)-2-cyclohexen-1-yl]amino]- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-*O*- $\alpha$ -D-glucopyranosyl-(1 $\rightarrow$ 4)-D-glucose), with mol wt 645.63, is an investigational drug. It is a pseudotetrasaccharide isolated from strains of *Actinoplanes* and contains an unsaturated cyclitol moiety.

Acarbose is a nonabsorbable  $\alpha$ -glucosidase inhibitor which blocks the digestion of starch, sucrose, and maltose. The digestion of complex carbohydrates is delayed and occurs throughout the small intestine rather than in the upper part of the jejunum. Absorption of glucose and other monosaccharides is not affected. Acarbose is administered orally three times a day and chewed with the first mouthful of food.

### Miscellaneous Agents

**Diazoxide** [364-98-7] (3-methyl-7-chloro-1,2,4-benzothiadiazine 1,1-dioxide) is a nondiuretic thiazide used for its hyperglycemic actions when given orally (Proglycem) and for its antihypertensive effects when given intravenously (Hyperstat I.V.) (see CARDIOVASCULAR AGENTS). Diazoxide produces a prompt dose-related increase in blood glucose by directly inhibiting insulin secretion and, possibly, by stimulating epinephrine secretion by the adrenal medulla (see EPINEPHRINE AND NOREPINEPHRINE). Diazoxide is used to counteract hyperinsulinism in conditions such as insulinoma. It is not indicated in the treatment of functional hypoglycemia.



# Diabetes, Insulin Resistance, Hyperinsulinemia and Vascular Disease: A Therapeutic Challenge for the 90s

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## Introduction

Diabetes mellitus is a complex metabolic disease in which the metabolism of carbohydrate, lipid and protein is significantly altered. The disease exists in two clinical forms: insulin dependent (IDDM) and non-insulin dependent (NIDDM) diabetes. IDDM accounts for 15-25% of the diabetic population of the United States, and is believed to be the result of autoimmune destruction of pancreatic islet  $\beta$ -cells [1]. These patients rely on the exogenous administration of insulin to sustain their lives. The vast majority of the estimated 15 million Americans with diabetes have the non-insulin dependent form. This heterogeneous disorder is characterized by impaired insulin secretion and insulin resistance [2-5]. In addition to diabetes, the insulin resistant state is also seen in non-diabetics who become overweight. Tissue sensitivity to insulin, in these individuals, decreases by about 35% when ideal body weight is exceeded by 35-40% [6-8]. In spite of a considerable impairment in the efficiency of insulin, glucose tolerance remains relatively constant in this group due to the ability of the pancreatic  $\beta$ -cells to hypersecrete insulin that offsets the resistance [9]. This compensation results in a marked hyperinsulinemia that can be observed in both diabetic and non-diabetic, insulin-resistant individuals. A body of evidence is accumulating in the literature relating hyperinsulinemia, insulin resistance and vascular disease. The purpose of this review is not to provide an extensive examination of those studies, but rather to highlight some of the salient data, and the therapeutic options available, as well as to point out opportunities for further drug discovery research.

## Insulin Resistance, Hyperinsulinemia and Vascular Disease

Himsworth was the first to describe insulin resistance in 1936 when he made the observation that many elderly and obese diabetic patients were relatively insensitive to the hypoglycemic action of insulin [10]. While the term insulin resistance is most commonly used to describe insulin's action on glucose homeostasis, this hormone has pleiotropic effects. Insulin resistance can, therefore, be described in terms of the biochemical and physiological changes that result from an impaired action of this hormone. Included in these effects are dyslipidemia characterized by hypertriglyceridemia [11-13], decreased high density lipoproteins (HDL) [14], and to a lesser extent, raised total and low density lipoprotein (LDL) cholesterol [14], hypertension [15-18], and a thrombotic diathesis associated with attenuated fibrinolysis [19,20]. Indeed, the clustering of insulin resistance, hypertension, hypertrig-

lyceridemia, and low plasma HDL cholesterol in the same individuals led Reaven to refer to them collectively as 'Syndrome X' [21].

Given the above findings, it is not surprising that individuals with NIDDM have a high incidence of premature atherosclerosis and increased morbidity and mortality from coronary artery disease, cerebrovascular disease and peripheral vascular disease [22,23]. It has been estimated that, despite the improvement over the years in diabetic patient care, the age-adjusted incidence of acute coronary events is six times greater in male diabetics and four times greater in female diabetics than in non-diabetics, independent of LDL cholesterol, HDL cholesterol, blood pressure and smoking [23].

## Epidemiology of Insulin Resistance and Vascular Disease

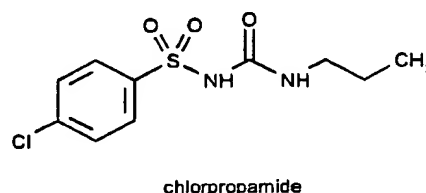
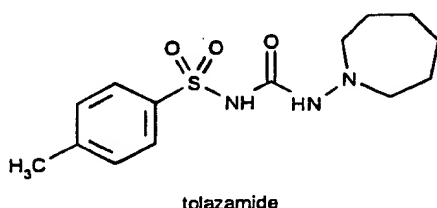
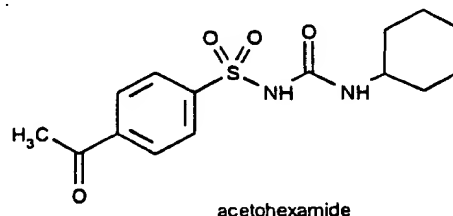
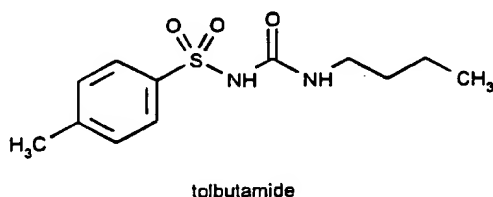
Several investigators have attempted to determine whether or not an association exists between the development of atherosclerosis and insulin resistance/hyperinsulinemia that is independent of the known risk factors of dyslipidemia, hypertension, hyperglycemia and obesity. Epidemiological studies have consisted of cross-sectional and prospective studies in both the general population and in diabetics [24]. While numerous studies have been carried out, a few should be discussed in particular because of the large numbers of patients included, the design of the studies, and the impact they have had on our current perceptions.

In 1985, Pyörälä and co-workers [25] published the results of a nine and a half year prospective study based on a cohort of 982 Helsinki policemen who were free of coronary disease at entry and were followed for the appearance of 'hard cardiac endpoints'. Multivariate analysis of the data showed that high plasma insulin 1 hr and 2 hr following an oral glucose load were found to be significant risk factors for the development of coronary heart disease (CHD) that was independent of other risk factors.

The Paris Prospective study examined fatal and non-fatal CHD in over 7000 male civil service workers, and reported results at 5 [26], 10 [27], and 15 years [28]. At the 15 year follow up, 2 hr post-load insulin remained a significant, independent predictor of death from CHD.

Recently, Solymoss and coworkers [29] studied 1119 French Canadians undergoing elective coronary arteriography for the evaluation of potential coronary artery disease. They examined the correlations between fasting serum insulin levels, the general features of insulin resistance, and various aspects of CHD. Patients were classified according to the quartiles of serum insulin. Those patients in the top quartile had an increased body mass index, serum triglycerides and decreased HDL cholesterol. Significantly more women in the fourth quartile had angiographically demonstrable significant stenosis of the coronary arteries, and previous myocardial infarction than those in the first quartile. There was a tendency for a

Figure 1. Sulfonylureas



greater incidence of previous myocardial infarction in men in the fourth quartile relative to the first quartile, but this did not reach statistical significance.

A number of cross-sectional studies have indicated that insulin treatment of NIDDM, and hence hyperinsulinemia, is strongly associated with CHD mortality [30, 31, 32], while a number of prospective studies have failed to find differences in cardiovascular disease morbidity and mortality as a function of insulin treatment. These include the University Group Diabetes Program (UGDP) [33] and the Diabetes Control and Complications Trial (DCCT) [34]. An excellent review of both of these seminal studies has been published [35]. The UGDP study compared diet therapy for NIDDM with standard insulin therapy and variable-dose insulin regimen, as well as administration of oral agents, on the incidence of cardiovascular and microvascular outcomes. The DCCT study, which examined the effects of conventional versus intensive management of IDDM on microvascular and neurological complications, was not primarily designed to test the effects of glucose control on the risk for macrovascular disease. The results of the UGDP study failed to show significant differences in cardiac or macrovascular endpoints when comparing the diet therapy group to the standard insulin or variable insulin therapy groups. After considerable sub-grouping and analysis of the data, the study committee concluded that, using a strict intention-to-treat criteria, no evidence exists that insulin therapy is superior to (or worse than) diet therapy alone for reducing the risk of vascular disease. The DCCT study on the other hand, showed far fewer cardiovascular events in the intensively treated group and a trend toward a reduction in the risk for cardiac and peripheral vascular events that failed to reach statistical significance [34].

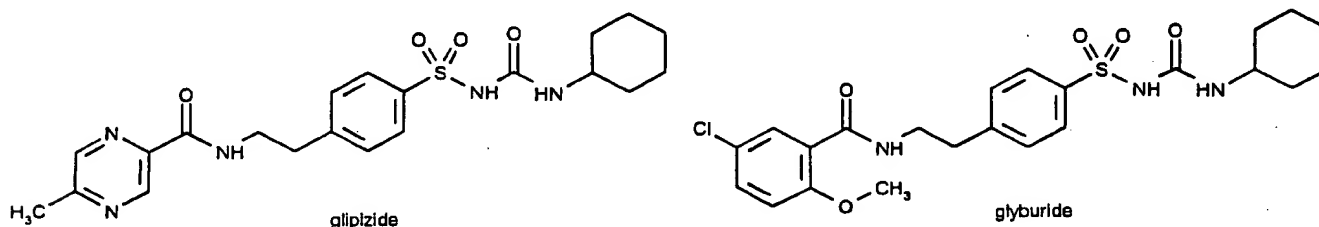
The evidence that these large, long-term, randomized studies provide does not resolve the issues relating to hyperinsulinemia, insulin resistance and vascular disease. Genuth [35] has provided some insight as to why definitive answers were not obtained. However, both of these studies were designed to test the glucose toxicity hypothesis. That is, hyperglycemia, or poor glucose control, leads to the

development of vascular disease. The proof for this hypothesis was not definitively obtained in the UGDP study, and the DCCT study was focused on microvascular events. However, it is also quite possible that glycemic control is only part of the pathogenetic process and that insulin resistance *per se* is a major contributor. None of the above mentioned studies made measurements of insulin sensitivity, and with good reason. Estimation of insulin sensitivity by the hyperinsulinemic, euglycemic clamp [36] is laborious and not suited to high throughput studies. In essence, the technique measures the rate of glucose disposal in individuals given a fixed, supraphysiologic level of insulin. As a physiologic equivalent, it might be quite useful to describe glycemic control in terms of the glucose-insulin ratio. This parameter would give an assessment of how much insulin is required to maintain the measured plasma glucose. Such a measurement would allow somewhat of an equalization of estimates of insulin sensitivity across a range of levels of glycemia. Additionally, it would provide an estimate of the level of insulin resistance to population based studies attempting to determine the role of that factor in the complications of diabetes.

### Pre-clinical Studies

In addition to the population studies implicating hyperinsulinemia in cardiovascular disease, a number of experimental findings have given some insight as to potential mechanisms for the role of this hormone in vascular disease. Insulin receptors have been identified in rat [37], bovine [38,39], and human [40] smooth muscle cells. It has also been shown to be mitogenic for these cells [41-44]. Insulin can stimulate lipid metabolism in smooth muscle cells from the rat [45] and can increase the binding of LDL to bovine smooth muscle cells [39] and human monocytes [46]. The activity of the rate limiting enzyme of cholesterol biosynthesis, 3-hydroxy,3-methylglutaryl coenzyme A reductase, is also increased in human monocytes by insulin treatment [47]. Several studies have attempted to examine the role of insulin in atherogenesis *in vivo*; these have been reviewed by Stout [48]. Most of the effects observed in these experiments have been

Figure 2. Second generation sulfonylureas



modest at best, with respect to the development of atherosclerosis. The literature is replete with references to the work of Duff [49,50] and McGill [51] regarding their observations concerning the role of insulin in the development of atherosclerosis in cholesterol-fed diabetic rabbits.

These investigators found that alloxan-induced diabetes would increase plasma cholesterol levels in rabbits, but decreased the arterial lesions in cholesterol fed rabbits relative to control, cholesterol-fed rabbits. Alloxan treated animals that failed to develop diabetes had the same degree of vascular lesions as control animals. When animals were treated with insulin, the degree of cholesterol deposition in the aorta of the diabetic rabbits was very similar to that of the controls [50]. However, recent work by Zilvermit and co-workers has demonstrated that the reason for this observation is probably related to impaired lipolysis of triglyceride-rich lipoproteins in untreated diabetic animals. This results in the formation of very large lipoproteins that are unable to enter the artery wall [52-55] and hence, cannot participate in the atherogenic process.

### Current and Emerging Therapies

The studies reported in the literature have not unequivocally implicated insulin resistance or hyperinsulinemia in vascular disease, nor have they exonerated it as a causal factor. In order to answer such questions, and to effectively treat NIDDM, it is necessary to have pharmacological agents that are capable of ameliorating insulin resistance without creating hyperinsulinemia. Several treatment options are available for controlling the metabolic abnormalities of NIDDM. Since obesity alone has been shown to cause insulin resistance and obesity is a major problem in NIDDM, a program of diet, weight loss and exercise can result in an improvement in insulin sensitivity without increasing plasma insulin levels [9]. Treatment with insulin in many cases will improve tissue sensitivity to the hormone. This enhanced sensitivity has been shown to improve markedly even when normoglycemia has been achieved by administering doses of over 100 units per day [56].

In lieu of insulin for the treatment of NIDDM, several orally active agents are available, including the sulfonylureas and metformin [57]. The sulfonylureas, (Figure 1), exemplified by tolbutamide, acetohexamide, tolazamide, and chlorpropamide, have been available for a considerable period of time. These agents stimulate the secretion of insulin in the absence of other secretagogues [58]. *In vivo*, their main effect is to sensitize the  $\beta$ -cell to glucose. They do not increase the synthesis of insulin. While the various sulfonylureas differ in pharmacokinetic

properties, there is no evidence that their mechanisms of action are different [59,73]. The so-called second generation sulfonylureas glipizide and glyburide (Figure 2) are the most potent agents in this class. They have been estimated to be 100 to 150 times more potent than tolbutamide on a molar basis [59,73]. The sulfonylureas are reasonably well tolerated. However, it has been estimated [57] that after 10 years, only about 50% of the patients initially started on this class of agents continue to have satisfactory control. Metformin, an agent recently approved for use in the United States and used for many years in Europe, is a member of the biguanide class of compounds (Figure 3). Other agents in this class include buformin and phenformin. Phenformin was used in the US for many years, but was withdrawn because of a high incidence of lactic acidosis [60]. Metformin has been used for a number of years in Europe. It appears to act by increasing peripheral and hepatic sensitivity to insulin without altering insulin secretion [61]. In comparison with sulfonylureas, this agent rarely causes hypoglycemia. Metformin's most common side effects are gastrointestinal. Lactic acidosis is rare, but can be fatal [57].

A relatively new class of agents, the thiazolidinedione derivatives (Figure 4), that improve peripheral insulin sensitivity are currently under development. Representative agents include ciglitazone, pioglitazone, englitazone, and troglitazone. These analogs have been shown to be effective in reducing hypoglycemia in various animal models including Zucker rats, ob/ob mice, and KKA<sup>y</sup> mice [62,63]. The hypoglycemic effects observed in the various animal models are associated with improvements in insulin sensitivity and without effect on insulin secretion. These agents do not produce hypoglycemia in the euglycemic state. Troglitazone, a compound in this class, is most interesting in that it contains the pentamethylhydroxychroman of vitamin E coupled to the thiazolidinedione. This gives the compound a certain amount of antioxidant activity that may be important in protecting LDL from oxidation [64]. Treatment with this agent completely prevented the development of insulin resistance, hypertension and hypertriglyceridemia in fructose-fed rats [65]. Troglitazone is not insulinomimetic *per se*, but does improve insulin sensitivity in streptozotocin-diabetic rats [66], as well as in normal animals [67].

Data concerning troglitazone are beginning to emerge from clinical trials. When 11 obese patients with NIDDM were treated with the drug for a period of 6-12 weeks, eight showed significant improvement in overall glycemic control, improvement in insulin sensitivity, and a reduction in plasma insulin levels [68]. In a double-blind, placebo-controlled study, treatment of diabetic patients with troglitazone over a three month period resulted in

Figure 3. Biguanides

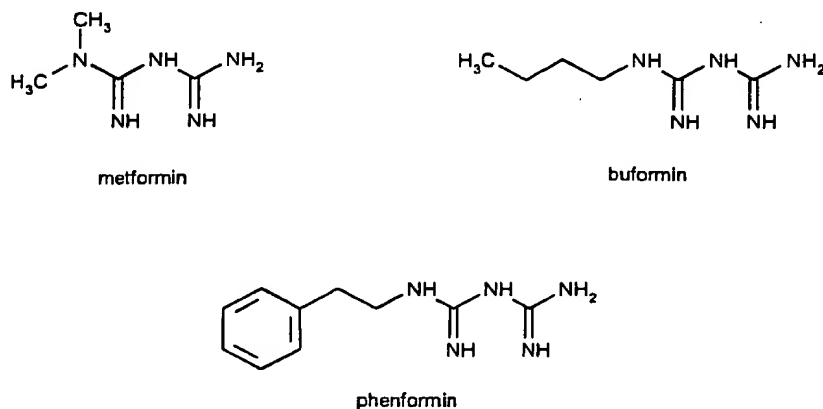
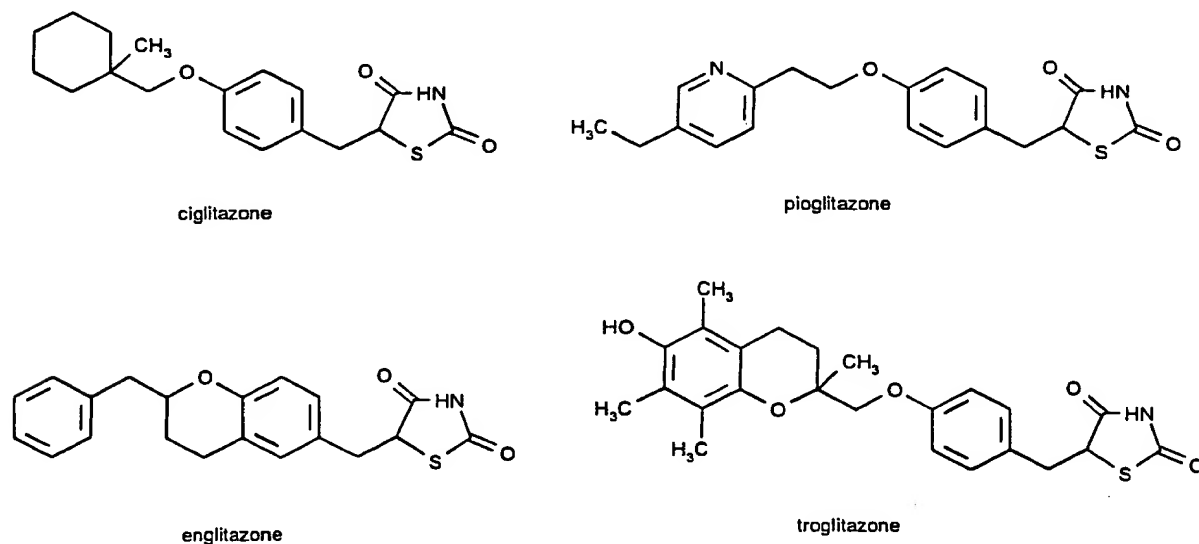


Figure 4. Thiazolidinedione derivatives



significant improvement in hyperglycemia, insulin resistance and plasma free fatty acid levels [69]. Iwamoto and coworkers [70] studied 19 NIDDM patients whose glycemic control was considered stable but unsatisfactory with diet and/or sulfonylurea therapy. Treatment with troglitazone resulted in significant improvement in glycemic control and insulin sensitivity without altering insulin secretion. The actions of this compound may also be important in preventing the onset of diabetes in obese individuals with impaired glucose tolerance. Eighteen non-diabetic, obese individuals were treated with either troglitazone or placebo for 12 weeks. Drug treatment resulted in improvement in glucose tolerance and a decrease in insulin resistance [71]. Ogihara and colleagues used troglitazone to treat 18 individuals with essential hypertension complicated by mild NIDDM [72]. An eight week course of therapy resulted in decreases

in fasting plasma glucose, fasting plasma insulin and a significant reduction in blood pressure. The reduction in blood pressure correlated significantly with the decrease in plasma insulin. These studies demonstrate that pharmacological intervention with troglitazone in NIDDM and in impaired glucose tolerance in obese individuals results in improvement in biochemical and physiological aberrations associated with insulin resistance and hyperinsulinemia, while simultaneously improving total body sensitivity to this hormone.

### Conclusions

The concept that insulin resistance and/or hyperinsulinemia are contributory factors for the development of vascular disease has been debated in the literature for almost 30 years. While the data are far from conclusive, a large body of laboratory and animal studies have

demonstrated a certain plausibility to the notion. The clinical and population based studies have been complicated by the confounding effects between hyperinsulinemia, obesity, dyslipidemia, hypertension and other classic risk factors. Also missing is some uniform, ready estimate of the degree of insulin resistance in any given individual or group of individuals. Calculation of the fasting or post-prandial glucose-insulin ratio may provide such a measure and help to clarify future studies.

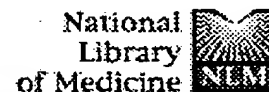
Most treatment options provide for glycemic control by inducing the secretion of insulin, or by providing pharmacologic doses of exogenous insulin. The biguanide metformin, and the advent of the thiazolidinedione class of 'insulin enhancing' agents promise to provide for glycemic control without stimulating insulin secretion, thereby reducing total exposure to this very potent hormone. The latter class of agents appear to be well tolerated, devoid of hypoglycemic liability, and have shown clinical promise under a number of experimental paradigms. The availability of such agents may provide very useful tools for the study of the involvement of insulin resistance and hyperinsulinemia in vascular disease.

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## Use of insulin-sensitizing agents in patients with polycystic ovary syndrome.

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Bloomgarden ZT, Futterweit W, Poretsky L.

Division of Endocrinology, Department of Medicine, Mount Sinai School of Medicine, New York, New York, USA.

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**OBJECTIVE:** To review the subject of polycystic ovary syndrome and the therapeutic use of insulin-sensitizing agents in patients with this endocrinopathy. **METHODS:** We present background information on this disorder and summarize the pertinent published literature. **RESULTS:** Polycystic ovary syndrome affects approximately 7.5% of reproductive-age women in the United States. Although specific diagnostic criteria for this condition have not been established, the presence of three major factors-chronic anovulation, hyperandrogenemia, and clinical signs of hyperandrogenism-has been proposed as essential for consideration of the diagnosis. A high ratio of serum luteinizing hormone to follicle-stimulating hormone is found in 60 to 75% of women with this syndrome. Treatment with metformin may yield heterogeneous responses in differing populations with polycystic ovary syndrome, but most studies have shown evidence of restoration of ovulatory cycling. In addition, weight loss and decreases in free and total testosterone levels have been reported. Troglitazone therapy proved somewhat less efficacious than metformin for restoring menstrual cycles and similar to metformin in producing hormonal responses. Because troglitazone is no longer available for clinical use, studies will need to be extended to other thiazolidinediones. Patients treated with another insulin sensitizer, D-chiro-inositol, have demonstrated improved insulin sensitivity, ovulatory rates, and biochemical findings. **CONCLUSION:** Current evidence suggests that the use of insulin-sensitizing agents in patients with polycystic ovary syndrome not only improves their sensitivity to the effects of insulin on glucose and lipid metabolism but also ameliorates clinical and biochemical manifestations of hyperandrogenism and increases rates of ovulation. Multicenter studies with larger numbers of patients are needed.


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**PPARgamma and atherosclerosis: effects on cell growth and movement.**

**Hsueh WA, Law RE.**

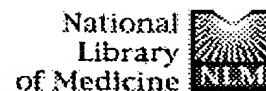
Division of Endocrinology, Diabetes and Hypertension, UCLA,  
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⇒ Atherosclerosis is a major vascular complication of diabetes and the primary cause of mortality in persons with this disease. Metabolic abnormalities related to the Insulin Resistance Syndrome or Metabolic Syndrome may importantly contribute to the increased risk of atherosclerosis associated with diabetes. Thiazolidinediones (TZDs) are oral insulin sensitizers in broad clinical use that enhance insulin-stimulated glucose uptake into skeletal muscle. TZDs can also improve cardiovascular risk factors and exert direct effects on vascular cells to potentially retard the atherosclerotic process. Direct vascular effects of TZDs likely result from their activity as ligands for the nuclear receptor, PPARgamma. All of the major cell types in the vasculature express PPARgamma, including intimal macrophages and vascular smooth muscle cells (VSMCs) in human atheroma. TZDs block VSMC growth by inducing cell cycle arrest in G1 through an inhibition of retinoblastoma protein phosphorylation. Migration of monocytes and VSMCs is also inhibited by TZDs, possibly through decreased matrix metalloproteinase production. Activation of PPARgamma by TZDs in macrophages induces ABCA1 transporter expression to promote reverse cholesterol transport. These antiatherogenic activities may also occur in vivo because TZDs have been shown to inhibit lesion formation in several animal models. Thus, TZD activation of PPARgamma may protect against atherosclerosis both by normalizing proatherogenic metabolic abnormalities of the insulin resistance/diabetes milieu and through an inhibition of vascular cell growth and movement.

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## Troglitazone improves psoriasis and normalizes models of proliferative skin disease: ligands for peroxisome proliferator-activated receptor-gamma inhibit keratinocyte proliferation.

Ellis CN, Varani J, Fisher GJ, Zeigler ME, Pershadsingh HA, Benson SC, Chi Y, Kurtz TW.

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Department of Dermatology, University of Michigan Medical School, Ann Arbor 48109-0314, USA.

**BACKGROUND:** Psoriasis is often treated with agents that activate nuclear hormone receptors for glucocorticoids, retinoids, and vitamin D. The peroxisome proliferator-activated receptor-gamma (PPARgamma) is related nuclear hormone receptor that can be activated by its ligands, including the thiazolidinediones. **OBJECTIVE:** To assess whether treatment with troglitazone, a currently available thiazolidinedione used to treat diabetes mellitus, has an effect on psoriasis in normoglycemic patients and whether ligands for PPARgamma have an effect on models of psoriasis. **DESIGN:** Open-label administration of troglitazone in patients with psoriasis and evaluation of drug actions in cellular, organ, and transplant models of psoriasis. **SETTING:** University and community hospital outpatient departments and university laboratories. **PATIENTS:** Patients with chronic, stable plaque psoriasis and control subjects. Five patients with psoriasis received troglitazone (none withdrew); 10 different untreated patients and 10 controls provided tissue samples. **INTERVENTIONS:** Oral troglitazone therapy at various dosages in patients with psoriasis; also, use of troglitazone, ciglitazone, and 15-deoxy-delta-12,14-prostaglandinJ2 in psoriasis models. **MAIN OUTCOME MEASURES:** Investigator-determined clinical results in patients and cell counts and histological evidence in models. **RESULTS:** All patients' psoriasis improved substantially during troglitazone therapy. Peroxisome proliferator-activated receptor-gamma was expressed in human keratinocytes; ligands for PPARgamma inhibited the proliferation of normal and psoriatic human keratinocytes in culture. Troglitazone treatment normalized the histological features of psoriatic skin in organ culture and reduced the epidermal hyperplasia of psoriasis in the severe combined immunodeficient mouse and human skin transplant model of psoriasis ( $P < .05$  compared with untreated controls). **CONCLUSIONS:** Peroxisome proliferator-activated receptor-gamma might be a useful intracellular target for the treatment of psoriasis; further study is needed to assess the clinical value of ligands for PPARgamma, including troglitazone.

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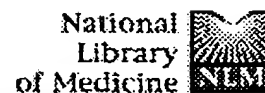
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**Thiazolidinediones inhibit osteoclast-like cell formation and bone resorption in vitro.****Okazaki R, Toriumi M, Fukumoto S, Miyamoto M, Fujita T, Tanaka K, Takeuchi Y.**

Related Resources

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Osteoblasts and adipocytes are derived from common bone marrow stromal cells that play crucial roles in the generation of osteoclasts. Activation of peroxisome proliferator-activated receptor-gamma (PPARgamma) induces adipogenic differentiation of stromal cells; however, whether this would affect osteoblast/osteoclast differentiation is unknown. Thus, we examined the effects of the thiazolidinedione (TZD) class of antidiabetic agents that activate PPARgamma on osteoblast/osteoclast differentiation using mouse whole bone marrow cell culture. As reported, all TZDs we tested (troglitazone, pioglitazone, and BRL 49653) markedly increased the number of Oil Red O-positive adipocytes and the expression of adiponectin and PPARgamma 2. 1alpha,25-Dihydroxyvitamin D3 [1,25-(OH)2D3] did not affect adipogenic differentiation induced by TZDs. TZDs did not affect alkaline phosphatase activity, an early marker of osteoblastic differentiation, despite their marked adipogenic effects. TZDs decreased the number of tartrate-resistant acid phosphatase-positive multinucleated osteoclast-like cells induced by 1,25-(OH)2D3 or PTH. Troglitazone dose dependently inhibited basal and 1,25-(OH)2D3- and PTH-induced bone resorption as assessed by pit formation assay. Interleukin-11 blocked the induction by troglitazone of adipogenesis, but had no effect on the inhibition of osteoclast-like cell formation. These results indicate that TZDs are potent inhibitors of bone resorption in vitro. Inhibitory effects of TZDs on osteoclastic bone resorption was not osteotropic factor specific and did not appear to be related to their adipogenic effects. Thus, TZDs may suppress bone resorption in diabetic patients and prevent bone loss.

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